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Bactericidal powers in the serum of healthy and diseased persons.

by E. G. Dresel and W. Keller.

Zeitschrift fuer Hygiene, 97: 151-161 (1922-1923).

In the course of studies of non-specific stimulant therapy by way of experimental increase in hemal anthracocides, Dresel and Freund (1) demonstrated that normally non-anthraxocidal human serum may develop anti-anthrax powers without the involvement of an immune process in the usual sense. It was found that an increase in anthracocidal substances may be triggered in rabbit blood by non-specific stimulant therapy as well as by pathological conditions or by pregnancy.

It was decided, therefore, to examine the bactericidal properties of human serum in connection with pathological conditions, during menstruation and in the newborn. As a sequence to the previous report, primary consideration was given to the reaction to anthrax bacilli; in addition, both anthrax and typhus bacilli were used in a number of serum tests, because Dresel and Freund had concluded that the effective substances in humans may originate in cells other than the blood platelets, i. e. platelets must not necessarily be involved exclusively.

Test procedure.

230 sera of healthy and diseased persons were examined as follows: Each serum, never more than 48 hours old, was distributed on 4 sedimentation tubes in amounts of 0.5 cc each. A broth dilution of 1:1,000 was prepared from a 24-hour sporeless, well-shaken anthrax bacillus broth culture, and one drop thereof was added to every sedimentation tube from a pipette of equal calibration. The first tube was immediately poured into an agar plate, the second after 1 hour of incubation at 37°C, the third after 4 hours, the fourth after 6 hours. Four sedimentation tubes per test furnished the control; they contained 0.5 cc broth and 1 drop of the anthrax dilution and were converted to agar plates after 1, 4 and 6 hours in the incubator.

The colonies were counted after 24 hours in the incubator. Incubation for 48 hours failed to produce a noteworthy increase in colonies, causing us to terminate the tests after 24 hours in every case.

The anthrax strain utilized, an old collection strain of the hygienic institute, was marked from the start by a low tendency to sporulation; furthermore, the anthrax threads were easily disseminated throughout the broth culture by agitation. When the strain failed to show characteristic growth on agar control plates after 1½ years of use and almost daily transfer, i.e. the colonies lacked the delicate marginal entanglements, a number of sera were tested with 4 additional, different anthrax strains. Of these, 2 strains had been grown from material obtained from diseased persons in 1912 and 1914, 2 originated with Siberian horsehair and Indian goat's hair. When all 5 strains were affected uniformly by the serum the last 100 sera were treated with strain III from Siberian horsehair. It also shows a slight lag in sporulation and is pathogenic for mice. The original strain I has completely lost its ability to form spores and did not regain it even after a mouse passage. It killed a white mouse inoculated at the root of the tail with 1 loopful of an agar culture after 60 hours, and did so even after being excluded from the investigation owing to its uncharacteristic growth (*).

It should also be mentioned that the preparation of broth and agar used in the tests were made with horse meat and that the hydrogen ion concentration was tested with the comparator according to Michaelis, in order to secure identical growth conditions. The anthrax strains proved quite sensitive to fluctuations in pH.

(*) In his paper, On the influence of intravenous instillation of protein substances on the bactericides of normal serum, O. Pfeiler (2) concludes that his observations of the effect of caseosane on the anthracocidal power of rabbit serum disagrees with the findings reported by Dresel and Freund. The following must be noted in this connection:

1. Our reports do not apply to rabbit serum, which invariably possesses strong anthracocidal powers, but to the increase in anthracocides in the plasma and in the fresh blood extract of rabbits following caseosane injection and other non-specific stimuli. For this reason alone the results do not permit comparison. Secondly, the test procedure used by O. Pfeiler seems questionable to us. His germ seeding figures fluctuate between 85,000 and 120,000 germs per cc of serum, whereas we never exceeded 2,000 germs per ½ cc, or 4,000 per cc of serum in the case of anthrax bacilli. Experience teaches that the results of bactericidal tests are closely related to the quantity sowed, since quantitative relations are involved. Moreover, we consider the technique of plate pouring used by us, involving as it does the counting of germs, superior in such investigations to the streaking method used by Pfeiler. The fact that anthrax strain "Heidelberg", when used by Pfeiler, was apathogenic for mice, can probably be explained only by the difference in the nutrients used in cultivation. Dresel and Freund.

In order to avoid over-burdening this publication with data, reference is made to Dresel and Freund's paper, showing detailed data on p. 327 ff., both negative and positive. Considering the large number of tested sera, only the germ count of the immediate broth control and, secondly, the lowest germ count produced by anthracocidal substances, will be listed here. The symbol 1,000/0 therefore means that the immediate germ count of the broth culture was 1,000, while the germ count in the serum tubes sank to 0, either immediately or after 1, 4 or 6 hours. In all cases where a complete destruction of all germs did not occur, the second figure represents the lowest germ count of the serum agar plates. This lowest count was found already after 1 or 4 hours in the majority of tests, not rarely after 6 hours. In these cases a uniform decrease was invariably noted. When the bactericidal powers of the serum were exhausted after 1 or 4 hours without killing all germs, the examination after 4 or 6 hours naturally yielded a renewed increase, although this increase regularly was left far behind the increase in bacterial growth in the controls. The tests with normal serum never gave evidence of such action of weaker anthracocides.

Tests with the serum of healthy persons.

In examinations of serum from men, consideration had to be given to possible after-effects of wartime typhus and cholera immunizations, although this was unlikely after the transpired interval. The sera of 12 clinically healthy men without recent histories of illness were examined. Seven of these had assuredly been inoculated against typhus and cholera during the war, one had not been immunized, the rest could give no reliable information. The sera of these 12 men contained no traces or only scant traces of anthracocidal substances. The sera of 5 youthful women were similarly constituted. Since Dresel and Freund had noted an increase in anthracocidal powers in the plasma of rabbits after bleeding, and since in the meantime anthracocidal elements had been demonstrated in the serum of women during the menses, care had to be taken that serum samples of women, in order to be classed as normal, were not incumbered with histories of immunization against typhus and cholera, or had been procured during menstruation. Four women had not been inoculated, all 5 were 2-10 days short of impending menstruation. All 5 had no or very slight anthracocidal powers in the blood.

Result: Clinically healthy men and women, the latter within the menstrual interval, have no or few traces of anthracocidal elements in the blood. Immunization against typhus and cholera of 3 years standing has no effect on the formation of anti-anthrax powers.

Serum of newborn.

Since we had demonstrated in the previous report that pregnancy in the last weeks evokes strong anthracoides in the serum of healthy women, it seemed promising to examine the serum of newborn for anti-bacterial powers. The serum samples were furnished by the obstetric clinic; the blood samples were obtained there from the umbilical vein during stricture of the umbilical cord. 22 sera were tested against anthrax, 16 of these also against typhus bacilli.

The bactericidal effect against anthrax was: 600/0 - 600/0 - 600/0 - 580/0 - 580/10 - 580/52 - 580/0 - 580/0 - 350/17 - 350/0 - 350/85 - 350/32 - 350/70 - 350/53 - 260/0 - 152/0 - 152/6 - 152/1 - 190/0 - 350/18 - 350/81 - 350/0.

The bactericidal effect against typhus was: 3,000/0 - 3,000/0 - 3,000/25 - 3,000/0 - 2,500/0 - 2,500/0 - 2,500/11 - 3,000/0 - 3,000/50 - 3,000/0; in 6 additional cases a very marked decrease was noted after the sowing of 3,000 germs.

Result: The serum of newborn contains large amounts of bactericidal substances against anthrax and typhus bacilli.

Serum during menstruation.

14 sera of clinically healthy, youthful female persons were tested, 5 of these on the first day of menstruation, 5 on the second, 1 on the fourth and 3 on the seventh day of menstruation.

The bactericidal effect against anthrax bacilli was: 1st day: 500/265 - 500/12 - 500/125 - 960/36 - 350/8; 2d day: 520/16 - 400/0 - 400/260 - 500/175 - 800/155; 4th day: 960/400; 7th day: 750/5 - 960/120 - 650/45.

The sera of 2 women were tested again 3 days prior to the menses and now did not contain anti-anthraxic substances. The results of these two cases during the menses are underlined.

Result: During menstruation, the serum of clinically healthy women contains large amounts of anti-anthraxic powers, which have disappeared in the interval shortly before menstruation.

Sera of clinically ill persons.

31 cases of different febrile, acute infectious diseases were examined. Four cases of pneumonia gave: 630/0 - 320/0 - 1,200/0 - 950/170 (10 days after defervescence only 1,200/560); 1 interlobular exudate: 600/0; 2 cases of grippe: 500/0 - 750/0; in addition, 3 sera of patients were tested who had recovered from a severe case of grippe.

Nr. 1 gave 960/0 4 weeks after recovery; Nr. 2 750/11 6 weeks after recovery; Nr. 3, 960/500 $\frac{1}{2}$ year after recovery; 1 case of sepsis: 630/0; four patients with an unexplained ward epidemic who had been admitted with another minor ailment and suddenly fell ill for a few days with fever up to 39.8°C: 620/0 - 340/0 - 349/0 - 950/4; 2 cases of scarlet fever: 500/60 - 1,200/0. A third case of scarlet fever had 860/80 during desquamation, after defervescence. One case of pyogenic dermatosis: 860/13. Polyarthritides rheumatica: 1; 470/0. Erythema exsud. after joint rheumatism: 1; 260/0; arthritis gonorrhoeica: 2; 880/0 - 1,200/0; the same patient after defervescence 1,200/0. In addition, one case without fever: 340/80. Parametritis: 1; 740/0. Cystitis and pyelitis: 7; 470/0 - 800/30 - 960/15 - 960/3 - 340/0 - 1,000/0 and one patient whose serum was tested three times in intervals of 8 days and 6 weeks: 600/0; 740/0; 240/0. Inflammatory processes following abortion: 2; 800/0 - 314/0. (These 2 cases and the following are not unequivocal, since pregnancy could be a factor). The first case involves an induced abortion in the third month, the second, an abortion with placental polypus in the 5th month. The next case, a febrile bronchitis, was in the 6th month of gestation: 1,200/15. A pleuritis exsudativa of unknown etiology: 1,200/0.

Result: 40 sera from acute febrile diseases revealed very strong anti-anthrax substances.

Sera of tubercular persons.

Three tubercular cases diagnosed clinically and by X-rays, but without elevated temperatures and without tubercle bacilli in the sputum, involving cirrhotic apical and upper-lobe processes, gave the following serum test results: 269/10 - 800/600 and 860/880. The first case showed a poor general state of health with low hemoglobin and secondary anemic symptoms, which probably explain the presence of anthracocidal substances.

Result: In connection with old, non-febrile, cirrhotic tubercular processes of the lung, anthracocidal powers may be absent or present in small amounts only.

In seven cases of tuberculosis with very low, usually early-morning temperature elevations of a few 1/10 degrees above 37°C with positive X-ray diagnosis, of which 2 had negative and 5 positive sputa, the serum test indicated: 600/400 - 340/160 - 1,000/220 - 800/250 - 1,200/770 - 800/4 - 500/1. Five of these seven persons were women from which blood was withdrawn during the menstrual interval. The last two cases with the huge divergence are not quite clear. The next to last patient had blood in the stool, but the question of intestinal TB or a fresh ulcer ventriculi could not be decided. The last patient had had a hemoptysis 4 weeks prior to withdrawal of blood.

Result: Open and arrested pulmonary tuberculosis with slight elevations in temperature may evoke a considerable increase in anthracocidal properties of the serum

In 11 cases of tuberculosis, the majority highly febrile, with positive bacillary findings in all cases, the serum test resulted in: 1,200/0 - 1,200/6 - 260/0 - 1/0 - 340/0 - 1,200/0 - 1,200/14 - 800/0 - 560/0 - 500/0 - 1,200/0. These included 4 women whose blood was drawn during the menstrual interval.

Three of these patients died within one week after the withdrawal of blood, three within 1½ months. All 11 cases were afflicted with severe disseminated tuberculosis. Four of the five still alive had only pulmonary involvement, one had peritonitis exudativa tuberculosa as a complication. Section of the deceased revealed pulmonary and laryngeal TB in one case, one case of pulmonary and intestinal TB, one case of pulmonary TB and general sepsis, one case of pulmonary, general glandular and genital TB, one case of pulmonary and peritoneal TB and one case of pulmonary and miliary TB.

Result: The serum of persons with disseminated tuberculosis and high fever contains copious anthracocidal powers. No conclusions may be drawn as yet concerning the relation between the anthracocidal properties of the serum and the extent or form of the disease.

Sera of syphilitic persons.

The serum test of 7 cases of syphilis without fever and without preceding anti-syphilitic treatment yielded: 190/0 - 630/140 - 1,100/650 - 620/0 - 340/0 - 560/0 - 620/60. All 7 cases had positive Wassermann reactions. Involved were 1 encephalitis luetica, 1 lues larvata, 1 lues hereditaria, 1 syphilitic glandular disease, 1 recent infection and 2 patients with heart disease without previous treatment. Five treated cases, 3 of them with mercurial and 2 with salvarsan therapy, partly dating back several years, yielded serum test results of: 600/0 - 620/9 - 340/0 - 1,600/600 - 620/120. The Wassermann reaction was positive in all cases. Two cases of tabes dorsalis with positive Wassermann, of which the first had received combined treatment in 1914 and in 1921, while the second had been ill and untreated for 1½ years, gave 2,000/12 - 1,000/5.

Two cases are particularly noteworthy; one that showed 1,100/650 without treatment and killed all 620 anthrax germs 2 hours after a salvarsan injection following completed mercurial therapy.

Another case of angina luica was tested 3 times. The Wassermann reaction was questionable. The first serum test yielded 340/0, the second after 10 days 860/5, the third after 14 additional days 800/300. 0.3 Neosalvarsan was injected between the second and third withdrawals of blood, 6 days prior to the last venipuncture.

Result: In syphilis, both untreated and treated, very large amounts of anthracocidal elements are found in the serum, apparently subject to fluctuation when subjected to treatment.

Anemias.

Serum tests of 2 patients with anaemia perniciosa gave: 1,000/0 - 1,000/0 - ; in a case of leukemia 960/0; in a case of pseudoleukemia 260/0. In 7 secondary anemias sequential to ulcus ventriculi: 1,000/0 - 800/220 - 960/240 - 750/2 - 751/1 - 630/0 - 340/0; in one following an ulcus duodeni 320/0. In 4 cases of carcinomatous cachexia the results were: 314/0 - 620/0 - 350/0 - 350/15. The serum of a case of pelvic tumor gave 350/75; in a case of sarcoma of the thigh, 350/7. A case of morbus Banti showed 260/70.

Result: Strong anthracocides are found in anemias and secondary anemias.

Rheumatic diseases.

In 3 cases of joint rheumatism with salicylic therapy the serum test revealed 2,000/0 - 630/1 - 630/1, in 2 cases without therapy: 190/0 - 340/0.

Result: Strong anthracocides are found in rheumatic inflammations of the joints wither without salicylic therapy.

Hepatic diseases.

Seven cases of hepatic disease were tested with the following results: Cirrhosis hepatis: 1; 460/1; icterus with dystrophia adiposo-genitalis: 1; 340/7; cholangitis: 1; 560/0; cholecystitis: 1; 314/2; cholelithiasis: 3; 340/0 - 560/10 - 325/65.

Result: Anthracocidal substances appear in the serum in hepatic diseases.

Chronic renal afflictions.

Three sera of chronic nephritides gave: 1,000/0 - 1,600/700 - 960/300. A case of uremia was tested 4 times. The first test gave 880/26, 5 days later 900/23, after 5 additional days 1,100/1,060, 3 days later 960/0. The reason for the nearly negative result of the 3rd sample is not clear. Perhaps the two large withdrawals of blood preceding were responsible, but this would not explain the appearance of strong anthracocides 3 days later.

Result: Anthracocides are present in chronic renal afflictions.

Chronic cardiac and vascular diseases.

The examination of the serum in a case of mitral insufficiency with stenosis yielded 400/75; a case of compensated vitium cordis in connection with a pulmonary gunshot wound, 1,000/28; a case of hypertonia with atherosclerosis, 1,000/1; a pericardiac abnormality with subfebrile temperatures,

960/226; in two cases of atherosclerosis with negative Wassermann 190/0 - 2,000/7; in a case of apoplexia with mitral stenosis and negative Wassermann, 560/200; in a case of dysbacia angiosclerotica, 740/45; in a case of severe misuse of nicotine and nicotine poisoning, 340/28.

Result: Copious anthracocidal substances appear in chronic cardiac and vascular diseases.

Chronic diseases.

The serum test in a case of chronic bronchitis produced 560/30; a case of chronic dysentery with rectal growths, 1,000/0; in another, 630/22; in a case of extended lumbago after over-exertion, 152/0; in a case of transverse myelitis after pararitium, 520/3; in spondylitis traumatica, 1,600/112; in two paravertebral abscesses, 620/20 - 560/15; in epididymitis gonorrh. 750/336.

Result: Anthracocides in copious amounts appear in chronic inflammatory processes.

Constitutional diseases.

Serum tests in a case of diabetes mellitus with chronic neuritis and gingivitis yielded 470/0; in a case of dystrophia adiposogenitalis, 740/0; a case of thyreoidism, 1,000/140; a case of constitutional eczema with eosinophilia, 560/350; in Jackson's epilepsy, 1,000/260.

Result: Moderate to large amounts of anti-anthracic elements are demonstrable in the serum in different constitutional diseases.

Various diseases.

A case of chronic cephalaea revealed 320/60, a case of chronic malaria with pleocytosis and dilatatio cordis, 1,000/690; chronic ischias under chinine therapy, 860/155; a case of gastropsis, 1,000/21. In addition, there were three neurasthenics, the first had a shock reaction with slightly elevated temperatures, the Wassermann was negative: 630/80; the second was an immoderate smoker and suffered from chronic angina: 560/1; the third failed to yield a diagnosis, his stomach had been evacuated the day before: 1,000/660.

In addition, the sera of three clinically healthy persons revealed strong anthracocidal properties: A physician with a femoral amputation with extensive muscular destruction at the stump suffered in the war: 750/5; a man who had suffered a severe wartime gunshot wound in his lung and had been subjected to an abdominal and appendicular operation a year ago: 2,000/4; a female physician (in her menstrual interval) who had undergone several major venipunctures for scientific research $\frac{1}{2}$ year ago: 960/175.

Disappearance of anthracocides.

The anthracocide induced by some kind of stimulus, such as acute or chronic infection with or without fever, or hemorrhage, or pregnancy, or degenerative and scarifying processes, seems to disappear after abatement of the stimulus.

An indistinct febrile infection with fever in excess of 39°C killed all of the anthrax bacilli introduced into the serum at the height of the fever, while 260 of 1,200 germs remained viable 14 days after defervescence. In a case of pneumonia that failed to kill 170 of 950 germs at the apex of fever, 506 of 1,200 germs remained viable after the abatement of fever.

On the other hand, an excessive stimulus due to an accumulation of morbid processes in the body or therapeutic effects, does not seem to produce the expected anthracocides.

The serum in a case of pernicious anemia with 2,780,000 red and 5,000 white cells which had been treated for 2 months with electroferrol and, in the last two weeks before venipuncture, daily with 3 X 0.05 arsacetine, failed to show anthracocides.

The serum of a female patient with chronic pulmonary tuberculosis, cholecystitis and cholelithiasis, whose blood sample was withdrawn during the period, killed only 100 of 800 anthrax germs introduced.

The serum of a case of severe uremia with retinitis did not contain anthracocidal substances.

In the case of a female patient with congenital syphilis and chronic arthritis with salicylic therapy in the 9th months of gestation, 900 of 1,200 anthrax germs introduced into the serum survived.

The serum of a case of angina luica gave the following result: The serum of the untreated patient killed 340 anthrax bacilli. Ten days later 5 of 860 anthrax germs survived, after 14 additional days (6 days after a salvarsan injection of 0.3) 300 of 860 bacilli remained viable.

The serum of a woman with severe diabetes mellitus (4.3% sacch. acetone and acetic acid ~~+~~), withdrawn on the second day of her menses, showed no anthracocides.

Action of the serum against anthrax and typhus bacilli.

Next, the question was to be clarified whether the bactericidal elements in the serum of healthy and sick persons are capable of killing other bacteria besides anthrax germs.

Fifty sera were tested simultaneously for bactericides against anthrax and typhus bacilli. Included were the sera of 5 ex-soldiers who had received repeated inoculations of typhus vaccine and those of 8 non-immunized men; 21 sera came from women, 9 from those past or short of menstruation, and 12 from women in the first or second day of the menses. Seven of these 12 sera came from clinically healthy women. The remaining 27 persons were non-ambulatory patients with clinical diagnoses. All sera revealed strong bactericidal properties against anthrax. Nineteen killed the bacilli completely. Thirty-two sera killed the typhus bacilli, fluctuating in count between 1,600 and 6,000; two showed a very marked decrease in the number of typhus bacilli introduced.

Serum from the umbilical vein of 16 newborn was tested against anthrax and typhus bacilli. As in the case of anthrax bacilli, the 3,000 typhus bacilli were completely inactivated in 7 cases; in three cases 11, 25 and 50 typhus germs survived; six cases indicated a very strong decrease in the number of typhus bacilli.

One is tempted to relate this result to other facts discovered so far in connection with the non-specific stimulant effect in the bodily fluids by means of biological, physical and chemical test methods. We deem it necessary, however, to collect additional evidence before hypotheses based on existing results are postulated.

Summary.

No anthracocidines are found in the serum of clinically healthy men and women in their menstrual interval.

The serum of clinically healthy women contains bactericidal elements against anthrax bacilli during menstruation, disappearing after 14 days.

The blood of the umbilical vein of newborn contains bactericidines against anthrax and typhus bacilli.

Sera of clinically sick persons contain copious amounts of anthracocidines in febrile, acute and chronic infectious diseases; in infectious diseases without fever (chronic tuberculosis, syphilis, rheumatism); in diseases of the blood; in chronic diseases with organic changes, such as hepatic afflictions, cardiac and vascular diseases and chronic renal affections; in serious injuries and operations with extensive scarifying processes.

Therapy may increase the formation of anthracocidines, but excessively large doses may also cause them to disappear. The coincidence of different, severe organic diseases seems to have the same effect. (Arndt-Schulz's law?). ----- Fifty sera killed both anthrax and typhus bacilli.

Literature: (1) Dresel, E.G. and H. Freund, Studies of non-specific stimulant therapy. 2d Report. On the experimental increase of anthracocidines in the blood. Arch.f.exp.Pathol.und Pharmacol. 91, H.6. - (2) Pfeiffer, Otto, On the effect of intravenous instillation of protein substances on the bactericide of normal serum. Dr.Diss. Munich 1922, R. Oldenbourg.